NTP
National Toxicology Program

Benzene Low Dose Inhalation Induced Hematotoxicity and Genotoxicity Phenotypes and Haplotype Association Analyses

John E. French, Ph.D.
National Institute of Environmental Health Sciences

NTP Board of Scientific Counselors
December 9-10, 2009

Benzene - human and rodent myelotoxin and leukemo/lymphomagen

- Hematotoxic at ≤1ppm
- Complex metabolism
- Two pathways
- Biomarkers of exposure
- Candidate gene association studies

Hematotoxicity in Workers Exposed to Low Levels of Benzene

Qing Lan,[1]* Luoping Zhang,[2]* Guilan Li,[3] Roel Vermeulen,[1]
Rona S. Weinberg,[4] Mustafa Dosemeci,[1] Stephen M. Rappaport,[5]
Min Shen,[1] Blanche P. Alter,[1] Yongji Wu,[6] William Kopp,[7]
Suramya Waidyanatha,[5] Charles Rabkin,[1] Weihong Guo,[2]
Stephen Chanock,[1,8] Richard B. Hayes,[1]
Martha Linet,[1] Sungkyoon Kim,[5] Songnian Yin,[3]
Nathaniel Rothman,[1]† Martyn T. Smith[2]†‡

1774 3 DECEMBER 2004 VOL 306 SCIENCE www.sciencemag.org

Evidence That Humans Metabolize Benzene via Two Pathways

Stephen M. Rappaport,[1] Sungkyoon Kim,[2,] Qing Lan,[3] Roel Vermeulen,[4] Suramya Waidyanatha,[2] Luoping Zhang,[1] Guilan Li,[5] Songnian Yin,[5] Richard B. Hayes,[3] Nathaniel Rothman,[3] and Martyn T. Smith[1]*

[1]School of Public Health, University of California at Berkeley, Berkeley, California, USA; [2]School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; [3]National Cancer Institute, National Institutes of Health, U.S. Department of Health and Human Services, Bethesda, Maryland, USA; [4]Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands; [5]Institute of Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention, Beijing, China

946 VOLUME 117 | NUMBER 6 | June 2009 · Environmental Health Perspectives

Carcinogenesis vol.30 no.1 pp.50–58, 2009
doi:10.1093/carcin/bgn249
Advance Access publication October 31, 2008

Large-scale evaluation of candidate genes identifies associations between DNA repair and genomic maintenance and development of benzene hematotoxicity

Qing Lan*, Luoping Zhang[1], Min Shen, William J.Jo[1], Roel Vermeulen[2], Guilan Li[3], Christopher Vulpe[1], Sophia Lim[1], Xuefeng Ren[1], Stephen M.Rappaport[1], Sonja I.Berndt, Meredith Yeager, Jeff Yuenger, Richard B.Hayes, Martha Linet, Songnian Yin[3], Stephen Chanock, Martyn T.Smith[1] and Nathaniel Rothman

Rationale and Background

- Benzene - human and rodent carcinogen
- ADME kinetics - significant differences in toxicokinetics that may correlate with toxicity
- The MOA of benzene-induced hematotoxicity and genotoxicity remain unclear
- Toxicity and cytogenetic alterations were observed in myeloid progenitor cells

Rationale and Background

- HAM approach is powerful and allows identification of potential SNPs associated with sequences that modify the toxicity phenotype
- HAM using genetically-diverse homozygous mice under experimental exposure conditions may prove to be more powerful than GWAS studies in humans
  - ✓ eliminates allele-allele interactions (epistasis)
  - ✓ defined experimental and environmental conditions
  - ✓ intermediate phenotypes anchored to a stressor

Key Issues

- Benzene toxicity is greatest to the rodent and human hematopoietic system
- Liver metabolizes the greatest mass of benzene
- Bone marrow and other tissues may also metabolize benzene to toxic metabolites at low exposure
- More benzene is expired unmetabolized after administration by the oral route (60%) than by inhalation (14%) at equivalent oral dose (mg/kg)

Key Issues continued….

- High exposure levels (≥ 200 ppm (6 h TWA) saturating to detoxification
- Between 5 and 50 ppm benzene (6 h TWA), there is no significant difference between urinary metabolites in mice
- HQ/MA to phenol ratio after inhalation exposure to ≤ 50 ppm (6 h TWA) is similar between mice and humans
- Individuals may metabolize inhaled benzene differently based upon toxicokinetic differences

Hypothesis

Genetic and epigenetic variation (SNP and/or structural) between inbred strains determine the inhalation exposure level dependent tissue specific metabolism of benzene and tissue-specific benzene toxicity.

Experimental Design

Design: (ACUC approved)

•Animals: 5 male and 5 female mice/strain (5 mice x 2 sex x 4 doses x 34 strains = 1360 mice)

•Exposure: 0, 1, 10, or 100 ppm inhaled benzene (Inhalation exposure shall be six hours ±T90 per dose day.

•Exposure Length: 28 consecutive daily exposures (5 d/wk for 5 wks plus 3 consecutive days of exposure)

•Test agent: Benzene (plus [13C]-benzene for determining benzene mass equivalents, metabolites, and hemoglobin adducts

Experimental Design continued…

- Study loaded by staggered starts to allow sufficient time for collection of tissues and fluids for quantitative measurements
- All mice will be anesthetized by CO2-oxygen for blood and tissue collection
- At a 5% confidence level, 90% statistical power requires using at least 5 mice/sex/exposure to measure at an effect size of at least 2

Status: In Progress at NIEHS Inhalation Facility

- ACUC approval for exposures to 34 strains
- 4 GC units purchased to monitor each Hazleton chamber (air, 1, 10, or 100 ppm to achieve 6 h TWA, ± 10% target)
- Pilot 28 d exposures to C57BL/6J, C3H/HeJ, and the B6C3F1/J hybrid are underway to test protocols, tissue sample collections, and quantitative measures of genotoxicity and hematotoxicity
- Strains will be loaded in cohorts to allow for time for sample collections within 16-18 h of the final exposure

Significance and Expected Outcomes

This 28-day benzene exposure will determine the effects genetic variation on low level benzene exposures on

- Hematotoxicity (absolute quantities of peripheral circulating cells by differential cell count and bone marrow hematopoietic lineage)
- Genotoxicity (micronucleus data by fluorescence cytometry) that simulate human population exposures for haplotype-phenotype association analysis.
- Determination of [13C]-benzene mass equivalents will allow correlations with oral toxicokinetic studies

Significance and Expected Outcomes continued...

- Benzene oxide and hydroquinone hemoglobin adducts for biomarker correlation with genetic variation
- Haplotype association mapping to identify QTLs and human orthologs for
  - ✓functionally validation *in vitro* and *in vivo*
  - ✓epidemiology of benzene exposed human populations.

Acknowledgements

NTP/NIEHS

- Dan Morgan
- Keith Shockley
- Mike Cunningham
- Grace Kissling
- Frank Johnson
- Suramya Waidyanatha

University of California at Berkeley

- Martyn Smith
- Steve Rappaport

Questions/Discussion